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Note

Synthesis and semisynthesis of α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranose octaacetate

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Synthesis of the glycan chains of N-glycoprotein antenna is one of the ongoing research programmes in our laboratories [1–5]. In Fig. 1 the structure of a typical oligomannose-type oligosaccharide is presented.

In the past, for the construction of this glycan chain partially substituted methyl and phenyl 1-thio-α-D-mannopyranoside derivatives and potential Man(α1–2)Man glycosyl donors have been prepared [3]. From Fig. 1 it is obvious that a large amount of the mannobiosyl donor is necessary for the final success. This fact and a recently appeared article [6] prompted us to reinvestigate how to easily prepare this important key compound.

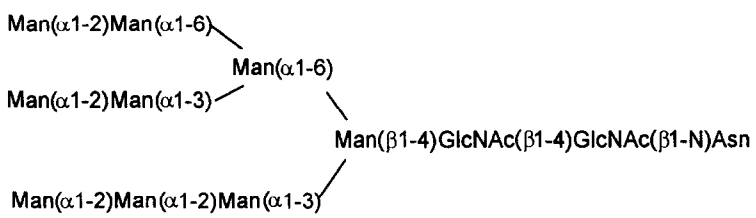

Fig. 1. Typical oligomannose-type N-glycan.

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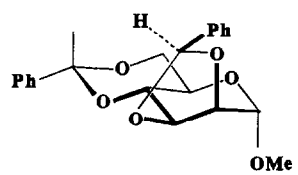
Comparing the synthetic routes based on phenyl 1-thio-α-D-mannopyranoside and the commercially available methyl α-D-mannopyranoside [3], the latter was selected for the preparation of the required potential glycosyl donor, and also for the synthesis of Man(α1–3)Man and Man(α1–6)Man.

It is well-known that methyl α-D-mannopyranoside (**1**), the cheapest mannose derivative, can be converted into a mixture of the *exo*-(**2**)- and *endo*-(**3**)-2,3:4,6-di-*O*-benzylidene acetals [7], and the large scale preparation of the highly crystalline **2** by fractional crystallization is not a problem. After chemoselective reductive acetal ring-opening of **2** with the lithium aluminium hydride-aluminium chloride reagent [8] the isolation of the major product **4** required column chromatography. In the first experiment, **4** was coupled with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**5**) using expensive silver triflate as the promoter [9] to give the disaccharide derivative **6** [3].

1



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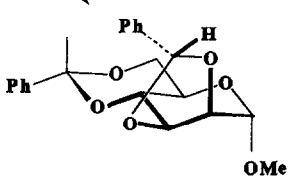

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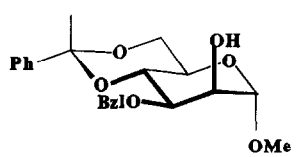

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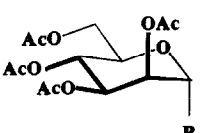
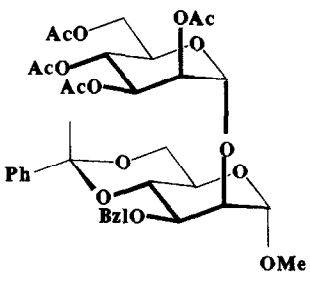
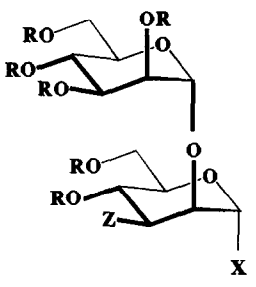


	R
5	Br
8	OH,H
9	$OC(=NH)CCl_3$

The reaction of **4** with **5** in the presence of the less expensive mercuric bromide and powdered molecular sieves [10] gave a complex reaction mixture from which **6** could be isolated in a poor yield (18%) by means of column chromatography. Acetylation of the by-products possessing lower R_f values and subsequent column chromatography yielded crystalline methyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-manno-pyranosyl)-α-D-mannopyranoside (**7**; 28%) indicating the hydrolysis of the acetal moiety during the long reaction time. For testing the imidate procedure [11] first compound **5** was reacted with water in dichloromethane in the presence of 'old' silver oxide giving 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose (**8**), which was converted into the corresponding imidate **9** [1] by the usual way. Coupling of the glycosyl acceptor **4** with **9** in the presence of trimethylsilyl triflate (0.3 equiv) and 4 Å molecular sieves (in pellet form) afforded **6**. Crystallization of the product and column chromatography of the mother liquor gave the disaccharide derivative **6** in 78% yield, being in the same range as the silver triflate promoted reaction. Compound **6** was converted into α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranose octaacetate (**10**) in a simple three-step procedure, including catalytic hydrogenation, acetylation and acetolysis [3]. Compound **10** is a potential glycosyl donor both for the Koenigs–Knorr reactions and for the imidate method [12,13]. Catalytical hydrogenation (Pd–C; H_2) and subsequent Zemplén deacetylation of **6** yielded methyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranoside (**11**). The physical data of **11** were in agreement with those reported in the literature [14].



6



	R	Z	X
7	Ac	OBzl	OMe
10	Ac	OAc	OAc
11	H	OH	OMe

All the chemical syntheses of mannobiose octaacetate **10** are expensive and time consuming. As an alternative we propose a novel semisynthetic route for obtaining this potential glycosyl donor starting from baker's yeast which is a rich source of manno-oligosaccharides. It should be noted that the partial acetolysis of isolated yeast mannan is a known procedure in the literature [15]. With the experiences on the acetylation and acetolysis of cyclodextrins in mind, we decided to apply this procedure on crude yeast mannan. Powdered baker's yeast was purchased in a local shop. Without isolation of the

mannan, this material (7 g) was acetylated at room temperature, and then acetolysed for 10 h at 70 °C with 10% sulfuric acid in acetic anhydride. After the workup procedure, the product was subjected to column chromatography on Kieselgel using 85:15 dichloromethane–acetone as the eluent, and 130 mg of compound **10** was isolated. A linear trisaccharide (200 mg) and higher oligosaccharides (290 mg) were also isolated and identification of these latter substances is in progress.

In conclusion, the most simple and cheap way to obtain a Man(α1–2)Man glycosyl donor seems to be the semisynthetic procedure using crude baker's yeast as a rich source of manno-oligosaccharides.

1. Experimental

General methods.—Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. NMR spectra were recorded with a Bruker WP-200 SY spectrometer. Reactions were monitored by TLC on Kieselgel $60F_{254}$ (Merck) with detection by charring with H_2SO_4. Kieselgel 60 (Merck) was used for short-column chromatography.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**6**).—*Procedure A.* A mixture of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**4**; 960 mg, 2.58 mmol) [8] and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**5**; 1.59 g, 3.87 mmol) in dry CH_2Cl_2 (20 mL), containing activated powdered 4 Å molecular sieves (3 g) was stirred under Ar for 15 min. Then, $HgBr_2$ (1.08 g, 3 mmol) was added and stirring was continued for 24 h. The mixture was diluted with CH_2Cl_2 (100 mL) and filtered through Celite. The filtrate was washed with aq 5% KI (2 × 20 mL) and water (3 × 20 mL), dried, and concentrated. The residue was subjected to column chromatography (85:15 CH_2Cl_2–EtOAc) to give **6** (326 mg, 18%); mp 142–143 °C (from EtOH); $[\alpha]_D$ +39.7° (*c* 0.65, $CHCl_3$); lit. mp 142–143 °C (EtOH); $[\alpha]_D$ +41.9° ($CHCl_3$) [3]. Further elution with 9:1 CH_2Cl_2–MeOH yielded a by-product, which was conventionally acetylated. The residue was purified by column chromatography (7:3 CH_2Cl_2–EtOAc) to yield methyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside **7** (505 mg, 28%); mp 99–100 °C (from EtOH); $[\alpha]_D$ +39.9° (*c* 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.32–7.23 (m, 5 H, Ph), 5.43–5.19 (m, 4 H, H-4,2′,3′,4′), 4.97 (d, 1 H, $J_{1',2'}$ 1 Hz, H-1′), 4.80 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.59 (s, 2 H, PhCH_2), 3.37 (s, 3 H, OMe), 2.14, 2.11, 2.09, 2.03, and 1.98 (5 s, 3,3,3,3,6 H, 6 Ac). Anal. Calcd for $C_{32}H_{42}O_{17}$: C, 55.01; H, 6.06. Found: C, 54.90; H, 6.09.

Procedure B. A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**5**; 822 mg, 2 mmol), 'old' Ag_2O (926 mg, 4 mmol), CH_2Cl_2 (4 mL) and water (0.2 mL) was stirred for 1 h at room temperature, then diluted with CH_2Cl_2 (40 mL) and filtered through Celite. The filtrate was washed with aq 10% $Na_2S_2O_3$ (10 mL) and water (3 × 10 mL), dried, and concentrated. To a solution of the residue in dry CH_2Cl_2 (8 mL) were added trichloroacetonitrile (2 mL, 20 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.3 mL, 2 mmol), and the mixture was stirred for 30 min, then concentrated. The crude product was purified by column chromatography (9:1 CH_2Cl_2–EtOAc containing 0.5% Et_3N) to yield **9** (610 mg, 62% based on **5**). Only the pure fractions,

free of UV active impurities, were collected; $[\alpha]_D$ +45.4° (*c* 0.62, $CHCl_3$); lit. $[\alpha]_D$ +45° ($CHCl_3$) [1]; +46.5° ($CHCl_3$) [16].

A mixture of **4** (372 mg, 1 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**9**; 591 mg, 1.2 mmol) in dry CH_2Cl_2 (10 mL), containing 4 Å molecular sieves (2 g; pellets) was cooled to −30 °C under Ar. A solution of TMSOTf (58 μL, 0.3 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise and the mixture was stirred for 30 min at −30 °C. After the addition of pyridine (1 mL) and CH_2Cl_2 (60 mL), the mixture was filtered through Celite, and the filtrate was washed with aq 5% $NaHCO_3$ (10 mL) and water (2 × 10 mL), dried, concentrated and co-concentrated with toluene (3 × 20 mL). The syrupy residue crystallized by treatment with an initial crystal, and then was recrystallized twice from EtOH to give **6** (345 mg, 49%); mp 142–143 °C; $[\alpha]_D$ +41.1° (*c* 0.95, $CHCl_3$). The combined mother liquors were concentrated and an additional amount of **6** (205 mg, 29%) was isolated by column chromatography (85:15 CH_2Cl_2–EtOAc). The overall yield of **6** was 78%.

Methyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranoside (**11**).—A mixture of **6** (246 mg, 0.35 mmol), EtOH (10 mL), HOAc (2 mL) and palladium on carbon (30 mg) was stirred in a H_2 atmosphere overnight. Then, the catalyst was filtered off, and the filtrate was concentrated and co-concentrated with toluene (3 × 10 mL). The residue was dissolved in dry MeOH (30 mL), NaOMe (catalytic amount) was added and the solution was kept at room temperature overnight. After neutralization with Amberlite IR 120 (H^+) resin and filtration, the solution was concentrated and the residue was passed through a column of Kieselgel 60 (2:1:1 BuOH–MeOH–H_2O) to give **11** (102 mg, 82%), isolated as an amorphous foam; $[\alpha]_D$ +62.4° (*c* 0.42, H_2O); lit. $[\alpha]_D$ +64.4° (H_2O) [14]; ^{13}C NMR (D_2O): δ 102.99 (C-1′), 100.05 (C-1), 79.2 (C-2), 61.7 (C-6,6′); FABMS: Calcd for $C_{13}H_{24}O_{11}$: 356.3. Found: 357 [M + H^+], 379 [M + Na^+].

Acetylation and subsequent partial acetolysis of baker's yeast.—Powdered baker's yeast (7 g) was suspended in Ac_2O (25 mL), and conc. H_2SO_4 (2.5 mL) was added in a period of 10 min, then the mixture was stirred for 10 h at 70 °C. After cooling it was poured into vigorously stirred warm water (500 mL). The solid separated was filtered off, washed with water (2 × 100 mL) and air-dried. The dry material was suspended in CH_2Cl_2 (300 mL), filtered, and the filtrate was washed with aq 5% $NaHCO_3$ (50 mL) and water (2 × 50 mL), dried over $MgSO_4$, and evaporated. The residue (3.6 g) was subjected to column chromatography on Kieselgel 60 (250 g) using CH_2Cl_2–acetone (9:1 → 85:15 → 1:1) as the eluent. Fractions having higher R_f values than the synthetic α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranose octaacetate were not collected. Fractions having the same chromatographic mobility as the synthetic material (R_f 0.49; 85:15 CH_2Cl_2–acetone) were collected to give **10** (130 mg); mp 139 °C (from EtOH); lit. mp 139–140 °C (from EtOH) [3]; $[\alpha]_D$ +35.3° (*c* 1.04, $CHCl_3$); lit. $[\alpha]_D$ +36.9° ($CHCl_3$) [12], +38.3° ($CHCl_3$) [3]; ^{13}C NMR ($CDCl_3$): δ 99.11 (C-1′), 91.31 (C-1), 75.77 (C-2), 62.29 and 61.58 (C-6,6′); ^{13}C NMR for synthetic **10**: δ 99.12 (C-1′), 91.30 (C-1), 75.76 (C-2), 62.30 and 61.60 (C-6,6′).

The major component of the acetolysis products having lower chromatographic mobilities was shown to be a linear trisaccharide (200 mg) with R_f 0.39 (85:15 CH_2Cl_2–acetone); $[\alpha]_D$ +41.5° (*c* 1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 99.77 and 99.25 (C-1′,1″), 91.45 (C-1), 62.40, 62.07, and 61.53 (C-6,6′,6″).

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